Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1973, **49**, 37-40

Arbovirus studies in Luanda, Angola

2. Virological and serological studies during an outbreak of dengue-like disease caused by the Chikungunya virus

ARMINDO R. FILIPE [1] & MANUEL R. PINTO [2]

An outbreak of dengue-like disease was observed in Luanda, Angola, at the end of 1970 and beginning of 1971. Chikungunya virus was isolated from the blood of a patient with typical symptoms of dengue and from a pool of Aedes aegypti *mosquitos. A survey for antibodies to arboviruses in the sera of persons living in Luanda showed that the Chikungunya virus was indeed responsible for the outbreak. The fact that this outbreak immediately preceded and continued concurrently with a yellow fever epidemic in Luanda shows that two arboviruses from different antigenic groups may circulate simultaneously in the same ecological area.*

The Chikungunya virus, which Ross isolated in Tanzania in 1956, has since been found in Zaire, Zambia, and South Africa (Taylor, 1967). In Angola, the occurrence of a dengue-like disease has been observed, but the etiology of the disease has never been demonstrated. However, serological studies carried out in 1960 by Kokernot et al. (1965) in Angola have shown that sera collected in the northern region of the territory had neutralizing antibodies to the Chikungunya virus.

Osterrieth et al. (1961) also reported an epidemic of dengue-like disease caused by this virus. The epidemic coincided with an outbreak of yellow fever in Doruma, Zaire, near the borders of Sudan and the Central African Republic.

During the last months of 1970 an outbreak of an infectious disease characterized by high fever, headache, and severe pains in the joints and muscles was observed in Luanda. The disease, known locally as Kâtolu Tôlu (Kimbundu dialect for " break-bone disease ") was observed before the yellow fever epidemic that started in January 1971. Cases continued to be seen until the end of the yellow fever epidemic at the beginning of April, when the vector was eradicated as a result of the intensive antimosquito measures taken by the public health services.

Two strains of arbovirus were isolated—one from the blood of a person with a typical dengue-like syndrome, obtained at the beginning of the disease, and the other from *A. aegypti* mosquitos captured in Luanda. A study of their properties showed both strains to be of the Chikungunya virus.

MATERIALS AND METHODS

Blood from several acute cases of the dengue-like disease was collected and the serum was inoculated by the intracerebral and intraperitoneal routes into 1–2-day-old Swiss mice of the Charles River strain.

The usual methods (Hammon & Work, 1964; Work, 1964) were used for recovering the virus from mosquitos. Fifteen pools of the mosquito species *A. aegypti*, *Mansonia africana*, *M. uniformis*, and *Culex fatigans* (Ribeiro, 1971) were captured in Luanda and its suburbs and were processed for virus isolation. A suspension in Hanks's solution with 0.4% of bovine serum albumin (Armour, Fraction V) was prepared from the triturated frozen mosquitos from each pool. The supernatant fluid of this suspension was administered to baby mice in the form of intracerebral and intraperitoneal inoculations. When the mice showed signs of disease, they were killed and a 10% suspension of their brains was made in the same diluent for further passages. Immune sera to the isolated viruses were prepared by inoculating this suspension by the intraperitoneal route into adult mice at weekly intervals for 5–6 weeks. An attempt was made to extract haemagglutinins from

* From the Institute of Hygiene and Tropical Medicine, Lisbon, Portugal.
[1] Research Virologist.
[2] Professor of Bacteriology and Virology.

the isolated strains by means of the sucrose–acetone method of Clarke & Casals (1958).

The human sera used for the survey for antibodies to arboviruses of group A were those collected in Luanda for the serological studies carried out during the yellow fever epidemic of 1971 (Pinto & Filipe, 1973). In all, 589 sera were studied by the haemagglutination (HI) test, following the techniques described by Clarke & Casals (1958), using the microtest with 4–8 haemagglutinating units and the following antigens from group A: Sindbis (Ar 339), Chikungunya (Ross), and O'Nyong Nyong.

In addition, 80 sera from persons suspected of having had the dengue-like disease were studied by the HI test. The 50 sera positive to the Chikungunya virus were further studied by the complement-fixation (CF) test with the Chikungunya antigen, also using the microtest.

RESULTS

Virological studies

Serum from a patient with typical " Kâtolu Tôlu ", collected 24 hours after the onset of the disease, yielded a virus (9-CT) pathogenic for baby mice. Some of the inoculated animals became sick and paralysed on the 4th or 5th day after inoculation of the serum; after the 4th passage, paralysis generally set in on the 4th day.

The supernatant fluid from a pool of 33 *A. aegypti*, inoculated by the intracerebral route into two litters of baby mice, yielded another virus (A-4) with pathogenic properties similar to those of the virus strain isolated from the blood of the above-mentioned patient.

Both strains were highly neurotropic for suckling mice; they rarely killed adult mice, although some of these developed alopecia, as has occasionally been observed in adult mice inoculated with Chikungunya virus or O'Nyong Nyong virus. Both strains were sensitive to sodium deoxicholate, with a sensitivity index of 4.4 log for strain 9-CT and 5.5 log for strain A-4.

It proved difficult to extract haemagglutinins from these strains; however, strain 9-CT yielded a haemagglutinin that could be used for the HI test. The optimum pH was 6.0.

The similarities between these two strains and the Chikungunya and O'Nyong Nyong reference viruses, as revealed by serological tests, are shown in Tables 1, 2, and 3. As a result of these similarities, it is difficult to differentiate the four viruses.

Table 1. HI titre [a] of the immune sera against the isolated strains and reference viruses

Immune mouse serum against:	Antigen		
	ONN	Chik.	9-CT
A-4 strain	0	160	—
9-CT strain	0	80	160
O'Nyong Nyong	160	40	0
Chikungunya	0	160	80

[a] Reciprocal of serum titre.

Table 2. Mouse neutralization test with the isolated virus

Virus	Antiserum	
	Chik.	ONN
A-4	2.6 [a]	3.4
9-CT	2.6	3.5

[a] Log neutralization index.

Table 3. Complement fixation tests with isolated and reference viruses [a]

Antigen	Antiserum			
	A-4	9-CT	Chik.	ONN [b]
A-4	16/16	16/32	16/16	8/16
9-CT	8/16	16/16	16/32	4/4
Chik.	32/8	16/32	16/32	16/16
ONN	16/8	8/8	8/8	32/128

[a] Reciprocals of serum dilution (numerator) and antigen dilution (denominator).
[b] Immune reference serum.

The neutralization indices of the acute and convalescent serum samples obtained from the patient from whom the 9-CT strain was isolated are given in Table 4. This shows a significant rise in the homologous neutralizing antibody, which neutralized also the Chikungunya and O'Nyong Nyong viruses.

Serological studies

The results of the investigation of sera for antibodies to arboviruses of group A (Table 5) show that only the Chikungunya antigen elicited a serological response. The results of HI testing of 80 sera from patients suspected of having had the dengue-

Table 4. Mouse neutralization test with sera from a patient

Serum	Virus		
	Chik.	ONN	9-CT
Acute	0	0.5	0.2
Convalescent	1.7 [a]	1.2	2.1

[a] Log neutralization index.

Table 5. Number of sera reacting with group A viruses (HI test) [a]

Serum	No.	Positive to Chikungunya	
		No.	%
Luanda residents	310	20	6.4
Family contacts of yellow fever cases	113	20	17.6
Yellow fever patients and suspected cases	166	41	24.6
Total	589	81	13.7

[a] All results for the Sindbis virus were negative.

like disease are shown in Tables 6 and 7. Of the 50 Chikungunya-positive sera subjected also to the CF test with Chikungunya antigen, only one was negative; another reacted at a low titre, and the others fixed complement at high titres. The results obtained with 20 of those 50 sera demonstrated that the infection by Chikungunya or an antigenically closely related virus was recent.

Table 6. Reaction with group A viruses (HI test) of sera from 80 patients suspected of having had "Kâtolu Tôlu"

Antigen	Serum	
	Positive	%
Sindbis	0	0
Chikungunya	52	65.0
O'Nyong Nyong	1	1.3
Chik. + ONN	27	33.7
Total	80	100

Table 7. Titres observed against Chikungunya and O'Nyong Nyong viruses

Titres	Positive for:			
	Chikungunya		O'Nyong Nyong	
	No.	%	No.	%
1/20	22	42	21	40
1/40	15	29	7	14
1/80	11	21	0	0
1/160	4	8	0	0
Total	52	100	28	54

DISCUSSION

It was not easy to identify the arbovirus strains isolated during the outbreak of dengue-like disease. The neutralization indices for these strains and the Chikungunya and O'Nyong Nyong viruses were similar, and CF testing with the homologous antisera did not allow the Chikungunya and O'Nyong Nyong viruses to be differentiated. However, HI tests of the homologous antisera showed higher antibody titres to the Chikungunya virus. These results are similar to those obtained by Williams et al. (1962) in their studies of the antigenic differentiation of the two viruses.

The results of the serological survey carried out in Luanda helped to elucidate the etiology of the disease. It is usually difficult to differentiate antibodies to the Chikungunya and O'Nyong Nyong viruses, as Henderson et al. (1970) pointed out when discussing the results of a survey for antibodies to arboviruses, carried out in Africa. However, the interpretation of the serological data obtained during a study of an epidemic of dengue-like disease does not seem to present the same difficulties. The results presented in Table 7 show that HI antibodies to the Chikungunya virus were always found in higher proportions and titres than those against the O'Nyong Nyong virus. A study of sera of Luanda residents with the disease showed that 65% reacted only with the Chikungunya antigen, 33.7% with the Chikungunya and O'Nyong Nyong antigens, and only 1.3% with the O'Nyong Nyong antigen alone. This reactivity was to be expected because of the antigenic overlap between the two antigens.

The results obtained in the study of sera positive to the Chikungunya antigen by the CF test indicated recent infection by this virus.

These data are supported by the epidemiological observations on the activity of the Chikungunya and O'Nyong Nyong viruses in Africa, which show that the Chikungunya virus has been endemic mainly in West Africa, whereas the O'Nyong Nyong virus has been endemic mainly in East Africa (Brès, 1970).

The simultaneous or consecutive appearance of two antigenically unrelated arboviruses having the capacity to survive and circulate in the same ecological area has been already observed in Africa with the Chikungunya and yellow fever viruses (Osterrieth et al., 1961) and in Asia with the Chikungunya and dengue viruses (Myers & Carey, 1967). The same phenomenon has now been reported in Luanda in 1971. This simultaneous presence of two arboviruses from different antigenic groups may add new dimenrions to the epidemiology of arbovirus diseases in stopical regions. Such epidemiological situations should be further investigated to elucidate their possible implications for public health in tropical countries.

ACKNOWLEDGEMENTS

We are grateful to the authorities of the Public Health Service of Angola for their cooperation; the Director and staff of the Institute of Scientific Investigation, Angola (IICA), Luanda, for laboratory facilities; Mr J. Teixeira dos Santos, Seca e Cruz and Mrs F. Dias Flora (IICA) and Mrs C. R. Bettencourt and Mr C. Gonçalves, Institute of Hygiene and Tropical Medicine, Lisbon, for their valuable technical assistance; Dr. H. Ribeiro, for classifying the mosquitos used in this study; and Dr N. Karabatsos, Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, Conn., USA, through whose courtesy the immune reference serum to the O'Nyong Nyong virus was supplied.

These studies were supported in part by the Institute of Advanced Culture (Instituto de Alta Cultura), Lisbon.

RÉSUMÉ

ÉTUDES SUR LES ARBOVIRUS À LUANDA (ANGOLA): 2. ÉTUDES VIROLOGIQUES ET SÉROLOGIQUES PENDANT UNE ÉPIDÉMIE D'UNE MALADIE DE TYPE DENGUE CAUSÉE PAR LE VIRUS CHIKUNGUNYA

A la fin de 1970 et pendant le 1er trimestre de 1971, on a observé à Luanda (Angola) plusieurs cas d'une maladie infectieuse bénigne, caractérisée par une fièvre élevée et de violentes arthralgies, qui a fait penser à une affection de type dengue. Cette épidémie a précédé et accompagné l'épidémie de fièvre jaune qui a sévi pendant les premiers mois de 1971.

On a isolé une souche de virus chikungunya à partir du sang d'un malade présentant la symptomatologie clinique classique de l'affection — désignée par la population locale par le nom de *Kâtolu Tôlu* (« os brisés »). Une autre souche du même virus a été isolée à partir d'un lot d'*Aedes aegypti* capturés à différents endroits de la ville de Luanda.

Simultanément, on a procédé à une enquête sérologique en utilisant les 589 sérums prélevés parmi la population de Luanda et de sa banlieue dans le cadre des investigations sur l'épidémie de fièvre jaune. Parmi ces sérums, 81 (13,7%) renfermaient des anticorps anti-chikungunya.

La présence simultanée de deux arbovirus appartenant à des groupes antigéniques différents, capables de survivre et de circuler dans le même contexte écologique, a déjà été observée en Afrique et en Asie. Ce phénomène peut ouvrir de nouvelles perspectives pour l'étude épidémiologique des affections causées par les arbovirus dans les régions tropicales.

REFERENCES

Brès, P. (1970) *Bull. Wld Hlth Org.*, **43**, 233

Clarke, D. H. & Casals, J. (1958) *Amer. J. Trop. Med. Hyg.*, **7**, 561

Hammon, W. McD. & Work, T. D. (1964) In: Lennette, E. H. & Schmidt, N. J., ed., *Diagnostic procedures for viral and rickettsial diseases*, 3rd ed., New York, American Public Health Association, p. 268

Henderson, B. E. et al. (1970) *Bull. Wld Hlth Org.*, **42**, 797

Kokernot, R. H. et al. (1965) *Trans. roy. Soc. trop. Med. Hyg.*, **59**, 563

Myers, R. M. & Carey, D. E. (1967) *Science*, **157**, 1307

Osterrieth, P. et al. (1961) *Ann. Soc. belge Med. trop.*, **41**, 207

Pinto, M. R. & Filipe, A. R. (1973) *Bull. Wld Hlth Org.*, **49**, 31

Ribeiro, H. (1971) *Rev. med. Angola*, **13**, 67

Taylor, R. M. (1967) *Catalogue of arthropod-borne viruses of the world*, Washington, D.C., US Government Printing Office (Public Health Service Publication No. 1760)

Williams, M. C. et al. (1962) *Trans. roy. Soc. trop. Med. Hyg.*, **56**, 166

Work, T. H. (1964) In: Lennette, E. H. & Schmidt, N. J., ed., *Diagnostic procedures for viral and rickettsial diseases*, 3rd ed., New York, American Public Health Association, p. 312